C L A I M S :

1. A method for identifying one or a small number of molecules, especially in a dilution of ≤ 1 μM, using laser excited FCS with measuring times ≤ 500 ms and short diffusion paths of the molecules to be analyzed, wherein the measurement is performed in at least one small volume unit of preferably ≤ 10^{-14} l or in a plurality of such a volume, by determining material-specific parameters which are determined by luminescence measurements of molecules to be examined.

2. A method according to claim 1, in which translational diffusion coefficients, rotational diffusion coefficients, excitation and emission wavelengths, the life of the respective excited state of a luminescing substituent, or combinations of these measuring quantities are determined as the material-specific parameters.

3. A method according to at least one of claims 1 and 2, characterized in that the space coordinates of the measuring volume are changed with respect to the space coordinates of the sample volume during analysis in order to analyze stationary or very slowly diffusing luminescing complexes by moving the sample volume with respect to the measuring volume and/or by changing the position of the laser beam and/or the position of the focus of the detecting optics with time wherein the measured translational diffusion coefficients correspond to a combination of the actual translational diffusion coefficients and the superimposed relative positional change of the coordinates of the measuring compartment.

4. A method according to claim 3, characterized in that the change of the coordinates of the measuring compartment with time defines an apparent diffusion time of a stationary or very slowly diffusing luminescing complex.

5. A method according to any of claims 1 to 4, characterized in that the luminescence of a substituent is directly interacting with the molecule to be determined wherein the substituent is a luminophorous ligand or a ligand complex the spectroscopic parameters of which are correlated with the type or function of the molecule to be determined.

6. A method according to at least one of claims 1 to 5 wherein the functional evaluation through the measurement of translational diffusion and/or rotational diffusion with evaluation of the correlation is performed particularly by determining the absolute number of molecules present and/or the variation thereof with time and/or by determining the specific concentrations of structurally distinct ligands and/or the ligand complexes and, derived therefrom, through the thermodynamic binding constants of specific interactions and/or the rate constants of specific recognition reactions or enzymatic processes involving ligand-coupled molecules.

7. A method according to at least one of claims 1 to 6 wherein the measured molecules or molecular complexes are of ionic or non-ionic nature.

8. A method according to at least one of claims 1 to 7, characterized in that the measurement takes place within a superimposed electric or magnetic field which is constant or varying with time.

9. A method according to claim 8, wherein the ionic molecules or molecular complexes of a sample volume are forced to pass through the measuring element or to remain in the

measuring element for a short period by means of a rectified electric field or an alternating electric field.

10. A method according to at least one of claims 8 and/or 9, characterized in that if an electric molecular trap is used, the labeled ligand bears a smaller charge than or a charge opposite to that of the target molecule to be complexed.

11. A method according to at least one of claims 1 to 10, characterized in that the analysis is coupled with an electrophoretic separation method to separate free dye-labeled ligands, especially nucleic acid probes, from specifically complexed ligands, especially nucleic acid hybrides wherein ligands with a multitude of coupled fluorescence dyes are preferably employed.

12. A method according to at least one of claims 1 to 11, characterized in that the complexes of labeled test reagent and analyte and possibly free analyte to be detected are preconcentrated in a first electrophoresis step and the complexes to be detected are transported into the measuring volume element in a second electrophoresis step.

13. A method according to at least one of claims 1 to 12, characterized in that the luminophorous ligand and/or the luminophorous ligand complex have an extinction coefficient of ≥ 30,000 with a quantum yield of ≥ 0.1 and/or one or more dye oligomers are used as the chromophorous ligand to shorten the effective measuring time.

14. A method according to at least one of claims 1 to 13, characterized in that the measuring compartments are arranged at an operating distance of ≤ 1000 μm from the emergence objective, the objective either being directly in contact with the sample volume or the sample volume being separated from the emergence lense by a transparent sheet.

15. A method according to at least one of claims 1 to 14, characterized in that defined molecules and/or equilibrium mixtures of molecules and/or kinetic reaction processes are analyzed in at least one sample volume element wherein in the case of several sample volume elements said elements are arranged on a two-dimensional carrier in two-dimensional arrangement, in particular on a membrane or sheet and/or wafer surface, or in a linear way, preferably in a capillary system, where the sample volumes preferably are present in or on natural cells or cells modified in vitro and/or in artificially prepared vesicular structures, particularly vesicles based on liposomes or based on soluble polymers having vesicle forming properties.

16. A method according to at least one of claims 1 to 15, characterized in that the individual sample volumes are generated by using a microdispensing system.

17. A method according to at least one of claims 1 to 16, characterized in that the access to phenotypically selected genotypes on DNA or RNA level is made possible by photochemical labeling of corresponding measuring positions, either by marking the local position of the respective volume element, preferably by employing the laser system used for the phenotype analysis using photochemically activatable substances for optical marking, or by enabling selective separation of the selected genotype by photochemically activatable reagents which are in contact with the contents of the selected volume elements in a soluble or surface-bound form and can enter a stable chemical interaction with structural elements of the selected genotype, especially by psoralen derivatives, and through specific binding to a coupled structural element, in particular activatable substances which are linked to oligonucleotides or biotin or avidin or streptavidin or oligopeptides or metal complexing agents or combinations thereof.

18. A method according to at least one of claims 1 to 17, characterized in that in a screening procedure substances that are possibly pharmacologically active are analyzed through their interaction with specific receptors by examining said interaction with binding of a luminescence labeled ligand to receptors wherein natural receptors on their carrier cells as well as receptors on receptor-overexpressing carrier cells or receptors in the form of expressed molecules or molecular complexes are used.

19. A method according to at least one of claims 1 to 18, characterized in that in the analysis of interaction of potentially active substances with specific receptors and a particular labeled physiological ligand at least two receptors are employed whose differential binding potential is determined through interfering binding of variants and a labeled natural ligand.

20. A method according to at least one of claims 1 to 19, characterized in that in the analysis of interaction of potentially active substances with specific receptors or intracellular substances of living cells within a sample volume the major part of the cells remains capable of dividing or metabolically active.

21. A method according to at least one of claims 1 to 20, characterized in that for the detection of specific recognition reactions the potential active substances are present in complex natural, synthetic or semisynthetic mixtures and said mixtures are subjected to chromatographic separation prior to analysis wherein the labeled ligand competing in the specific recognition reaction preferably is added to the separated fractions after the chromatographic separation and subsequently the competition reaction is analyzed in the specific interaction with a target molecule.

22. A method according to at least one of claims 1 to 21, characterized in that instead of the chromatographic unit a sample dispensing unit is employed.

23. A method according to at least one of claims 1 to 22, characterized in that the type and/or number of homologously complementary nucleic acid molecules in a sample volume is analyzed with at least one labeled nucleic acid probe through hybridization wherein the probe-coupled dye labels either preferably will not interact with a possible secondary structure of the probe or are preferably capable of specific intercalation and thereby change their spectral fluorescence behavior, especially by using substituted thiazole orange dyes.

24. The method according to claim 23, characterized in that as the labeled probes for the detection of nucleic acids single-stranded nucleic acids as excess components with a particular polarity (+ or - strand) in the form of synthetic or cellular RNAs or DNAs are preferably used..

25. A method according to at least one of claims 23 and/or 24, characterized in that the reaction rate of complex formation in hybridization is accelerated by performing the assay in a medium containing chaotropic salts and/or organic solvents, particularly phenols.

26. A method according to at least one of claims 23 to 25, characterized in that the degree of complementarity of the hybridized nucleic acid is analyzed through the thermodynamic stability of the complex.'

27. A method according to at least one of claims 23 to 26, characterized in that the detection of a complementary nucleic acid is quantified through using an internal standard, said internal standard differing from the sequence of the nucleic acid to be quantified in at least

one point mutation and the analysis being performed at a temperature at which the different conformations of the complexes of probe with internal standard and of probe with the nucleic acid molecule to be analyzed are distinct with respect to translational diffusion and/or rotational diffusion of the dye molecule.

28. A method according to at least one of claims 1 to 27, characterized in that the sensitivity for complex formation with dye-labeled ligand is enhanced by an increase of the effective association rate by adding to the reaction medium specific reactants which reduce the effective reaction volume and/or change the hydration sheath around the reactants and/or result in effective concentration of the reactants by phase separations, in particular by employing polymers and/or oligomers, preferably polyethylene glycols, dextranes, proteins, polyvinylpyrrolidone, chaotropic reagents, organic solvents.

29. A method according to at least one of claims 1 to 28, characterized in that the complex to be detected is made to react by at least one additional ligand which is preferably added in excess in order to increase the difference in size and/or shape between test reagent and the complex with the analyte to be detected.

30. A method according to at least one of claims 1 to 29, characterized in that the specificity of the detecting reaction is increased by performing the formation of an at least ternary complex with a combination of test reagents which preferably are in excess with respect to the analyte wherein at least one test reagent is labeled with at least one dye ligand the emission wavelength of which is apt for excitation of a fluorescence dye at a second test reagent which dye is coupled at least as a monomer, the fluorescence of which dye is detected.

31. A method according to at least one of claims 1 to 30, characterized in that at least two analytes are analyzed in one sample together in one assay through the reaction of two different test reagents which are labeled with at least two independant and different dyes and are either excited with light of different wavelengths or independantly detected by light of different emission wavelengths.

32. A method according to at least one of claims 1 to 31, characterized in that a particular analyte is complexed with at least two test reagents simultaneously which are each labeled with at least two optically distinct fluorescencing molecules wherein the simultaneous complex formation is detected either through the formation of an energy transfer complex and/or through the correlation in time of the signals having different wavelengths of excitation and/or emission.

33. A method according to at least one of claims 1 to 32, characterized in that samples are analyzed for mixtures of vesicular structures, in particular for lipid bearing vesicles, especially vesicles of the VLDL, LDL and/or HDL types, by staining the vesicles with fluorescence labeled antibodies and/or by incorporating fluorophorous label molecules specifically and permanently into the vesicular structures.

34. A method according to at least one of claims 1 to 33, characterized in that products of an in vitro protein biosynthesis are analyzed for specific binding properties or enzyme properties.

35. A method according to at least one of claims 1 to 34, characterized in that oligomer or polymer distributions are analyzed for average translational diffusion coefficients and/or average rotational diffusion coefficients and their respective half widths in the distribution.

36. A method according to at least one of claims 1 to 35, characterized in that in a sample volume with immobilized structure, preferably consisting of fixed cells or cell associates, tissues, organells, gel structures and other three-dimensionally compartmentalized sample volumes, several volume elements are covered with respect to dynamics or reaction kinetics of particular molecules wherein the positional coordinates of the volume elements are considered as well and the volume elements are subsequently assembled to a two- or three-dimensional image.

37. A method according to at least one of claims 1 to 36, characterized in that complex formation between receptor molecules and the ligands which are possibly present in a sample is analyzed in competition with dye-labeled ligands in solution or with involvement of solid phase coupled molecules or with involvement of cell-associated molecules, the biological samples being presented in sheets so that the measuring compartments to be analyzed are analyzed in a preferred distance of no greater than 1000 μm.

38. A method according to at least one of claims 1 to 37, wherein kit systems are used that have been developed for assays for the detection of fluorescence depolarization.

39. The use of a method according to claim 36 for the two- or three-dimensional imaging of dynamic processes or reaction kinetic processes in a three-dimensionally structured sample volume, preferably consisting of fixed cells or cell associates, tissues, organells, gel structures.

40. The use of the method according to at least one of claims 1 to 39 for in situ analysis of surface fixed preparations, in particular macromolecular complexes such as chromosomes, transcription complexes, translation complexes, or cells or tissue structures with the object of localization of particular target molecules and/or spatial correlation to

reference positions, preferably by using at least two ligands labeled according to the invention.

41. The use of the method according to at least one of claims 1 to 38 for the determination of reaction efficiency of a specific materials conversion or a binding reaction of a dye ligand bearing molecule by simultaneously or successively exposing to reaction conditions volume elements to be evaluated and by performing analysis of the reaction products after a defined reaction period.

42. The use of the method according to at least one of claims 1 to 38 for qualitatively and/or quantitatively covering specific molecules and/or molecular complexes and/or the molecular environment of the molecules and/or molecular complexes, in particular for the measurement and/or evaluation of physiologically active receptors, especially surface receptors or evaluation of receptor binding ligands or ligand complexes.

43. The use of the method according to at least one of claims 1 to 38 as an alternative for radioimmunoassays or enzyme linked immunoassays by measuring the competitive binding to a receptor molecule of a sought molecule with a luminophor bearing ligand.

44. The use of the method according to at least one of claims 1 to 38 for the analysis of complex molecular collectives such as replicative molecules, in particular nucleic acids and proteins or peptides derived therefrom, complex chemical reaction products, complex systems of products synthesized in chemical reactions, or complex mixtures of secondary metabolites as products of cellular synthesis.

45. The use of the method according to at least one of claims 1 to 38 wherein analysis of complex mixtures of substances is performed on line with analytical fractionating.

46. The use of the method according to at least one of claims 1 to 38 for the determination of the mobilities of molecules, molecular complexes, or cells, in particular of spermatozoa, monocytes, contractile elements, actively or passively transported molecules and membrane molecules.

47. A device for performing the method according to at least one of claims 1 to 38 with microscope optics known per se for laser focusing for the excitation of fluorescence in a small measuring compartment of a very dilute solution and for confocal imaging of the emitted fluorescence light for subsequent measurement wherein at least one system of optics with high numerical aperture of preferably ≥ 1.2 N.A. is employed, the light quantity is limited by a confocally arranged pinhole aperture in the object plane behind the microscope objective, and/or the measuring compartment is positioned preferably at a distance of up to 1000 μm from the observation objective.

48. The device according to claim 47 for the generation of diffraction limiting focusing of a laser beam with a unit generating the measuring signal and an observation unit wherein at the side of generation of the measuring signal an appliance (20) for prefocusing a laser beam (21), a dichroitic mirror (30) for deflecting said laser beam (21), and an additional lens (40) for focusing the laser beam onto the measuring volume are provided and wherein the observation unit has photon counting appliances (52), a correlation appliance (71), and a multichannel scaler appliance (72), and the measuring signal is optionally processed and/or evaluated in a computer assisted way.

49. The device according to claim 48, wherein the appliances (20) for prefocusing are provided with a lens (22) and an array (23) corresponding to microscope optics wherein a collineated laser beam (21) is focused on the image plane

B_1 by a lens L and on the image plane B_2 (first image) by said array (23).

50. The device according to claim 49, wherein said array (23) is provided with an exchangeable arrangement of lenses for the variation of the diameter of the prefocused laser beam (21).

51. A device according to at least one of claims 48 to 50, wherein a detection unit is constituted by two detectors (53, 54) with a beam splitter (60) partitioning the light (55) emitted from the sample to the detectors (53, 54).

52. The device according to claim 51, wherein the emitted light beam (55) passes imaging lenses (56, 57) and filter elements (58, 59) prior to each of the detectors (53, 54).

53. A device according to at least one of claims 48 to 52, wherein the detectors (53, 54) detect light of different wavelengths.

54. A device according to at least one of claims 48 to 53, wherein one or more detector elements are placed in the image plane optionally in the form of a detector array.

55. A device according to at least one of claims 48 to 54, wherein a pinhole aperture (50) is arranged in the beam path (55).

56. A device according to at least one of claims 47 to 55, characterized in that two objectives are used which form an angle of > 90° between them.

57. A device according to at least one of claims 47 to 56, characterized in that continuous lasers having emitted wavelengths of > 200 nm are used as the light source, in particular argon, krypton, helium-neon, helium-cadmium

lasers or lasers pulsed with high frequency of ≥ 20 MHz with a power of ≥ 0.5 mW.

58. A device according to at least one of claims 47 to 57, characterized in that appliances for single photon counting such as avalanche diode detectors are arranged in the beam path of the emitted light, preferably in the plane of the pinhole aperture, for detecting the emitted light wherein signal analysis is performed by a digital correlator or multichannel counter.

59. A device according to at least one of claims 47 to 58, characterized in that the measuring compartment is fixed in a sample volume between two capillaries, said capillaries being provided with a chemically inert conductive coating at the outer side, in particular metal vapor deposited coating, especially gold vapor deposited coating on a chromium priming, wherein the conductive coatings are connected with a computer controlled rectified field or an alternating electric field and are conductively connected with each other through the measuring compartment.

60. A device according to at least one of claims 47 to 59, characterized in that two microscope optics facing each other enclose the measuring compartment.

61. A device, in particular according to at least one of claims 47 to 60, characterized in that an electrophoretic additional device is provided having at least one electrophoresis cell having at least one opening for charging/discharging of the sample to be analyzed and/or a washing solution, a wall electrode, a ring electrode, a Neher capillary, an electrode at the tip of the capillary and a droplet outlet.

62. The device according to claim 61, characterized by an electric trap having a quadrupole element with at least

four electrodes, preferably pin electrodes or vapor-deposited electrodes in a wafer configuration wherein preferably a hole of < 1 mm is lined, preferably in combination with at least two additional electrodes in at least a sextupole arrangement wherein the quadrupole element is preferably provided with alternating voltage and a direct voltage is applied to the sextupole electrodes such that the polarity thereof is opposed to the charge of the molecules to be analyzed.

63. A device according to at least one of claims 61 and 62, characterized in that the sheet for receiving the samples has specific binding properties for molecules due to molecular derivatization, in particular in the form of ion-exchange ligands or affinity ligands, especially oligopeptides, polypeptides, proteins, antibodies or chelating agents, especially iminodiacetic acid or nitrilotriacetic acid ligands, particularly sheets having different molecular structures of different binding specificity as ligands in specific positions.

64. A device according to at least one of claims 61 to 63, characterized in that the sample volume is fixed on a sample receiving device which is two- or three-dimensionally controllable wherein the sample preferably can be fixed in defined space coordinates with respect to the measuring optics by using two- or three-dimensional piezo drives.

65. A device according to at least one of claims 61 to 64, characterized in that said device is equipped with an appliance which deflects the laser beam in defined coordinates and/or can definitely determine the position of focusing.

66. A method for the detection and/or identification of single microorganisms, particularly bacteria, suspensed cells or viruses, wherein specific interactions or enzymatic activities of fluorescence labeled target molecules with sur-

face-expressed structural elements of natural or genetically recombined membrane proteins are detected as the identification criteria.

67. The use of the method according to claim 66 for the detection and preparative recovering of at least one specific gene of a microorganism whose at least one gene product is presented at the inner or outer membrane or envelope.

68. The use of the method according to claim 66 for the determination of the functions of gene products of defined gene segments.

69. A method according to claim 1 for the identification of one or more molecules in several small volume units, characterized in that several small volume elements are analyzed as the measuring volumes out of a common larger or several small excitation volumes and/or are analyzed successively with or without changing the space coordinates of the measuring volume or volumes within the sample.

70. A device for performing said method using a multiarray detector having optics for illuminating an excitation volume comprising all or several measuring volumes or having an optical system for the parallel illumination of several excitation volumes.

71. A device for the detection of one or more molecules, molecular complexes and/or molecular fragments, in particular in a dilution of $\leq 1\ \mu M$, in small measuring volumes of preferably $\leq 10^{-14}$ l by means of fluorescence spectroscopy which device comprises

- a laser beam generation device for the generation of a laser beam with a first wavelength,
- a focusing device for the focusation of said laser beam onto the measuring volume wherein the laser beam is such highly focused within the measuring volume

that in essence it exclusively covers the measuring volume,

- a detector device for detecting fluorescence radiation generated due to the laser light excitation of one or more molecules, molecular complexes and/or molecular fragments, and
- a pinhole aperture arranged in the object plane within the beam path of the fluorescence radiation confocally with respect to the measuring volume to limit the quantity of fluorescence radiation to be detected by the detector device wherein said pinhole aperture has a diameter of especially ≤ 100 μm and preferably of ≤ 20 to 30 μm.

72. The device according to claim 71, characterized in that with an image scale of 1 : 100, 1 : 60, or 1 : 40 between the measuring volume and the object plane and with a measuring volume having dimensions of ≤ 0.1 μm in each direction, said pinhole aperture has a diameter of about 10 μm, 6 μm, or 4 μm, respectively.

73. A device according to claim 71 or 72, characterized in that the optics for the laser beam and/or the optics for the fluorescence radiation has a high numerical aperture of preferably ≥ 1.2 N.A.

74. A device according to any of claims 71 to 73, characterized in that the measuring volume is distanced from the focusing device by up to 1,000 μm.

75. A device according to any of claims 71 to 74, characterized in that the focusing device has a prefocusing device to prefocus the laser beam and a focusing objective lens to focus the prefocused laser beam onto the measuring volume.

76. The device according to claims 74 and 75, characterized in that the distance between the focusing objective lens and the measuring volume is up to 1,000 μm.

77. The device according to claim 75, characterized in that between the prefocusing device and the focusing objective lens a semitransparent mirror is arranged to deflect the prefocused laser beam onto the focusing objective lens.

78. The device according to claim 77, characterized in that the pinhole aperture is arranged at the side of the semitransparent mirror facing away from the focusing objective lens.

79. A device according to any of claims 71 to 78, characterized in that the detector device has at least one and preferably more detectors for detecting the fluorescence radiation.

80. A device according to any of claims 71 to 78, characterized in that between the pinhole aperture and the detector device, there is arranged at least one optical filter and/or at least one imaging lens and/or at least one semitransparent mirror and/or at least one reflecting mirror.

81. A device according to any of claims 71 to 80, characterized by an additional laser beam generation device for the generation of an additional laser beam with a wavelength different from the first wavelength, an additional focusing device for focusing said additional laser beam onto the measuring volume in such high an extent that the additional laser beam in essence exclusively covers the measuring volume, an additional detector device for the detection of fluorescence radiation generated due excitation of one or more molecules, molecular complexes and/or molecular fragments, and a correlator unit which is connected with the two detector devices.

82. The device according to claim 81, characterized by

- a T-shaped support with a first supporting arm (65) and a second supporting arm (74) connected therewith and running perpendicular to the first supporting arm (65),
- holding devices (83, 84) arranged at the ends at the faces of the second supporting arm (74) for axial guiding and holding of optical elements (lens, filter, mirror, detector) for the two laser beams and the two fluorescence radiations wherein the focused laser beams impinge on a glass slide bearing the measuring volume and being separably arranged preferably halfway between the two ends at the faces of the second supporting arm (74) and held by them,
- wherein the two holding devices (83, 84) can be moved synchronously relative to their respective ends at the faces of the second supporting arm (74) in the direction of the longitudinal extension thereof, the two holding devices (83, 84) are extended in the direction of extension of the first supporting arm (65), and the two laser beams can be deflected by deflecting mirrors and/or semitransparent mirrors (66, 67, 72, 73) through optical openings (69) out of the inside of the first supporting arm (65) onto the optical elements for the laser beams held at the holding devices (83, 84).

83. The device according to claim 82, characterized in that the optical elements for the laser beams are arranged at the inner sides, facing each other, of the two holding devices (83, 84) and the optical elements for the fluorescence radiation are arranged at the outer sides, facing away from each other, of the two holding devices (83, 84).

84. A device according to claim 82 or 83, characterized in that one of the focusing objective lenses can be positioned by an adjusting element which is in particular piezoelectric-

ally driven for compensation of an offset of the focuses of said focusing objective lenses.

add B'

add D1

add E4

add G16